



Research article

Seed germination inhibitory effect of *Caryota urens* L. seed pericarp on rice and associated weeds

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Abstract: Previous studies have shown that the *Caryota urens* seed pericarp possesses botanicals capable of inhibiting seed germination. Extracts made from *C. urens* pericarp were tested at different concentrations to find out its inhibitory activity. Three rice varieties (Bg 305, Bg 358, Bg 368) and the rice weeds (*Echinochloa crus-galli*, *Ischaemum rugosum* and *Ipomoea aquatica*) were tested. Percentage germination was measured for 7 days at 2 day intervals. The methanol extract of dried seed pericarp significantly reduced the seed germination, indicating that the concentration of the inhibitory substance/s in *C. urens* pericarp is higher in methanol extracts than the water extracts. Dried seed pericarp showed the highest inhibitory effect on the seed germination. The germination of all rice cultivars and weed species tested were completely inhibited by 100 and 200 mg.ml⁻¹ concentrations, suggesting that the *C. urens* seed pericarp has a seed germination inhibitory effect on all weeds tested. In general, the germination inhibitory effects were maximal at high concentrations than at lower concentrations. Potential for using *C. urens* pericarp for weed control is highlighted.

Keywords: *Caryota urens* - Seed pericarp - Germination - Rice - Weeds.

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INTRODUCTION

The ever increasing demand for food with the exponential increase in human population demands maximal achievements in crop production. Development of effective and environmental friendly weed control measures is one area of importance in this respect. Weeds and weed control have become a major cost factor determining the economic profitability of crop production worldwide. Weeds, being the major biotic stress for most crops including rice, compete with crops for light, nutrients and moisture, resulting in significant decrease of yield and quality of crop harvest. In the light of the above, studies on locally available natural sources that have a potential to control weeds become relevant to develop appropriate, environmental friendly control measures that suit the agricultural background and economy of Sri Lanka.

Allelopathy is defined as a mechanism by which plant, directly or indirectly affects, inhibits or stimulates growth of other plants by the production of chemical compounds or allelochemicals released to the environment (Ridenour & Callaway 2001). The use of allelochemicals by allelopathic plants/plant parts for weed management has received attention in recent times (Weston 1996) in view of their environmental friendly nature as opposed to synthetic chemicals. Hence the use of natural substances from plants is considered as a low input and sustainable approach to integrated weed management, a practice that helps reduce the increasing incidences of herbicide resistance in weeds as well (Mayer & Mayber 1989, Materechera & Hae 2008).

Studies have shown the inhibitory effects of certain plants not only on weeds but also on growth and yields of crop species. The allelopathic and herbicidal effectiveness of different plant species have shown to depend on the plant part (Oudhia 2003). Therefore investigations are required to explore plants and respective plant parts with effective allelopathic activity, especially in the control of agricultural weeds (Materechera & Hae 2008). The inhibitory effects of extracts obtained from different seed pericarps and plant parts on seed germination of

various other crops and weeds have been studied by Humaid & Warrag (1999), Kivi *et al.* (2010).

The study reported here is based on the seed germination inhibitory effect of *Caryota urens* (Family: Areaceae) seed pericarp. In *C. urens* the inflorescence is about 3 m in length and emerges at each leaf node from top to bottom. The plant produces pendent clusters of white, unisexual flowers resulting in about 35000 to 40000 seeds per inflorescence. Mesocarp is fleshy, filled with abundant irritant, needlelike crystals. When these fruits fall on the ground, it takes a long time to germinate. Therefore, it can be assumed that the seed pericarp of *C. urens* may contain seed germination inhibitory substances (Wijesinghe 1992). No study is known to have been conducted related to the seed germination inhibitory effects of *C. urens* seed pericarp on rice and associated weeds.

As no information is available on the pattern of *C. urens* seed germination, Ranasinghe *et al.* (2008) conducted an investigation to study the *C. urens* seed germination pattern and to develop a method for induction of rapid germination. In their study, it was found that the complete removal of fleshy pericarps of ripe *C. urens* fruits is the most effective way to achieve a higher rate of germination. The prevention of *C. urens* seed germination may be due to the presence of inhibitory substances in their pericarps which may contain relatively high concentrations of growth inhibitors that can suppress germination of the embryo (Taiz & Zeiger 2010).

This research was designed to study the inhibitory effect of *C. urens* seed pericarp on rice and on some associated weeds. *Echinochloa crus-galli* (L.) P.Beauv. is a grass weed that can germinate and grow for extended periods of time in an anaerobic environment (Kennedy *et al.* 1983) which is ecologically similar to rice. Being a highly competitive weed with rice, it can reduce rice yield up to 100% (Nyarko & Datta 1991). *Ischaemum rugosum* Salisb. is an annual grass weed that grows up to 120 cm in height. This aggressive, highly competitive weed is propagated by seeds. Five plants per square meter reduced 15% rice yield, while 80 plants per m² reduced 82% rice yield in a study conducted by Nyarko & Datta (1991). *Ipomoea aquatica* Forssk. is a fast growing, perennial broad leaf vine propagated by seeds and stem cuttings, and can cause up to 30% rice yield loss (Nyarko & Datta 1991). The three lowland rice cultivars, Bg 305, Bg 358 and Bg 360 tested have been developed by the Rice Research and Development Institute in Bathalagoda (Bg) in Sri Lanka (Personal communication, 18 September 2013).

MATERIALS AND METHODS

Selection of seeds

Three rice cultivars (Bg 305, Bg 358 and Bg 368) and the rice weeds (*Echinochloa crus-galli* (L.) P.Beauv., *Ischaemum rugosum* Salisb. and *Ipomoea aquatica* Forssk.) were selected for the experiment.

The inhibitory effects of the aqueous extract of Caryota urens seed pericarp on seed germination

Ripe fruits of *Caryota urens* were crushed and the pericarp was separated, homogenized and filtered. The extract was freeze dried. Lipolyzed seed pericarp extract (WESP) was used in germination inhibition experiments. A concentration series (0.5, 5, 50 and 500 mg.ml⁻¹) was made with distilled water. 2000 µl of this concentration series was added to the Petri dishes containing 20 seeds of test species on filter papers separately. Three replicates were used for each concentration. Distilled water was used in control experiment. During the experimental period, seeds were treated with distilled water. Number of germinated seeds and lengths of roots, shoots of germinated seedlings were recorded. Results obtained on percentage germination, root length and shoot length of each treatment were statistically analyzed.

The inhibitory effect of the methanol extract of Caryota urens seed pericarp on seed germination

Seed pericarps were oven dried for one week at 50 °C. Dried pericarps were immersed in methanol for a week and filtered through a muslin cloth. Methanol in filtrate was evaporated by rotator evaporation and the crude was freeze dried to remove excess water. The crude obtained from dried seed pericarps (DSPE) was stored in a freezer at -12°C. Seed germination inhibitory effect was observed using this DSPE crude. In the bioassay on filter papers, a concentration series (0.5, 5, 50 and 500 mg.ml⁻¹) from the DSPE was applied separately into Petri dishes containing 20 seeds of test species. 2000 µl of the extract was added per Petri dish. The dishes were kept wet by adding distilled water during the experimental period. Three replicates were used for each treatment. Distilled water was used for the control experiment. Germination of seeds, the length of shoots and roots of germinated seedlings were recorded. Results of each treatment were statistically analyzed.

Bioassay on soil

In the bioassay on soil, a higher concentration series (25, 50, 100 and 200 mg.ml⁻¹) was applied separately into

the Petri dishes with paddy soil containing 20 seeds of test species. 5000 µl of the extract was added per Petri dish. The soil was kept wet by adding distilled water, and the number of germinated seeds in each Petri dish was recorded daily. Three replicates were used for each treatment and distilled water was used for the control experiment. Percentage inhibition, shoot and root length of seedlings of each treatment were statistically analyzed.

RESULTS

Germination inhibitory effect of water-extracted seed pericarps on Echinochloa crus-galli seeds

Reference to table 1, *E. crus-galli* seeds in the control experiment were started to germinate on the 2nd day, and reached a percentage germination of 84.6% after six days, whereas the germinability of seeds treated with 500 mg/ml of WESP was significantly lower ($p < 0.05$) compared to the control. However, the other concentrations of WESP (50, 5 and 0.5 mg.ml⁻¹) did not show a significant difference ($p > 0.05$) in germination. In comparison with the control and other concentrations, a significantly ($p < 0.05$) shorter root length (4.8±2.3 mm) after 6th day was observed at 500 mg.ml⁻¹ WESP treatment. The lowest shoot growth ($p < 0.05$) was observed with the 500 mg.ml⁻¹ WESP treated *E. crus-galli* seeds (5.9±2.0 mm), whereas the shoot length of the control was 27.6±2.0 mm after six days.

Table 1. Effect of water extract of *Caryota urens* pericarp on germination of *Echinochloa crus-galli*.

Concentration (mg.ml ⁻¹)	Germination (%) ± SE	Mean Root length (mm) ± SE	Mean Shoot length (mm) ± SE
500	47.1±3.3 ^c	4.8±2.3 ^c	5.9±2.0 ^c
50	73.8±3.2 ^{ab}	18.1±2.3 ^a	16.3±2.0 ^b
5	77.3±3.4 ^a	21.4±2.2 ^a	22.1±2.2 ^a
0.5	79.6±3.3 ^a	30.3±2.3 ^a	26.9±2.0 ^a
Control	84.6±3.3 ^a	26.2±2.3 ^a	27.6±2.0 ^a

Note: Values are means of three replicates ± standard error with twenty seeds each. Values in a column with the same superscript are not significantly different ($p > 0.05$).

Germination inhibitory effect of methanol-extracted seed pericarps on Echinochloa crus-galli seeds

The seeds of *E. crus-galli* in the control began to germinate on the 2nd day resulting in a percentage germination of 80.5% after six days, whereas the germinability of seeds treated with 500 mg.ml⁻¹ and 50 mg.ml⁻¹ of DSPE were significantly lower ($p < 0.05$) compared with the control (Table 2). The other concentrations of DSPE (5 and 0.5 mg.ml⁻¹), showed a significantly lower ($p < 0.05$) germination rate (Table 2). A significant ($p < 0.05$) shorter root lengthening was also observed after the 6th day in 500 and 50 mg.ml⁻¹ DSPE treatments. Even the other two treatments, 5 mg.ml⁻¹ and 0.5 mg.ml⁻¹ reduced the root growth (Table 2). In the case of shoot development, a significantly lower shoot growth was observed in all 500, 50 and 5 mg.ml⁻¹ DSPE treatment, except in the 0.5 mg.ml⁻¹ treatment where no significant ($p > 0.05$) effect was found in the shoot elongation (Table 2).

Table 2. Effect of methanol extract of *Caryota urens* pericarp on germination of *Echinochloa crus-galli*.

Treatment (mg.ml ⁻¹)	Germination (%) ± SE	Mean Root length (mm) ± SE	Mean Shoot length (mm) ± SE
500	19.0±3.7 ^c	0.9±2.9 ^c	0.7±2.1 ^c
50	28.3±3.5 ^c	3.2±2.7 ^c	2.8±2.1 ^c
5	66.6±3.4 ^b	18.0±2.8 ^b	16.6±2.2 ^b
0.5	68.3±3.7 ^b	31.8±2.9 ^a	27.2±2.1 ^a
Control	80.5±3.3 ^a	43.1±2.8 ^a	32.9±2.0 ^a

Note: Values are means of three replicates ± standard error with twenty seeds each. Values in a column with the same superscript are not significantly different ($p > 0.05$).

Effect of DSPE extract on rice seeds and other associated weeds on filter papers

Reference to Table 3, 0.5 and 5 mg.ml⁻¹ treatments showed a significant inhibition in germination of Bg 305, Bg 358 and Bg 360 rice seeds, while 50 and 500 mg.ml⁻¹ treatments completely inhibited the seed germination of Bg 305, Bg 358 and Bg 360. Compared to the control 0.5 mg.ml⁻¹, 5, 50 and 500 mg.ml⁻¹ treatments inhibited the seed germination of *I. rugosum*. Among 0.5, 5 and 50 mg.ml⁻¹ treatments, the highest inhibitory activity was observed with the 50 mg.ml⁻¹ concentration, whereas 100% inhibition was observed in 500 mg.ml⁻¹ treatment. A

significant inhibition in germination was observed in *I. aquatica* seeds compared to its control. Both 50 and 500 mg.ml⁻¹ treatments completely inhibited the germination of *I. aquatica* (Table 3).

Table 3. Effect of the DSPE on the three rice cultivars and the three rice weeds.

Concentration (mg.ml ⁻¹)	Percentage inhibition				
	Bg 305	Bg 358	Bg 360	<i>I. rugosum</i>	<i>I. aquatica</i>
Control	63.3±1.7 ^a	7.5±1.1 ^a	22.5±1.1 ^a	45.8±2.4 ^a	49.2±3.7 ^a
0.5	74.2±2.4 ^b	12.5±1.1 ^b	53.3±1.7 ^b	68.3±3.3 ^b	78.3±4.0 ^b
5	76.7±1.7 ^b	12.5±1.1 ^b	40.8±1.5 ^c	66.7±3.8 ^b	79.2±5.2 ^b
50	100 ^c	100 ^c	100 ^d	95.0±2.2 ^c	100 ^{bc}
500	100 ^c	100 ^c	100 ^d	100 ^c	100 ^{bc}

Note: Values are means of six replicates ± SE, each with twenty seeds. Values in a column with the same superscripts are not significantly different (p>0.05).

Effect of DSPE extract on rice seeds and associated weeds on soil

The 25 mg.ml⁻¹ treatment did not inhibit the germination of Bg 305 rice seeds significantly (Table 4). But the 50 mg.ml⁻¹ treatment showed a significant inhibition of germination, while the 100 and 200 mg.ml⁻¹ treatments completely inhibited the Bg 305 seed germination. In the case of Bg 358, no significant difference was observed in seed germination treated with 25 and 50 mg.ml⁻¹ concentrations, whereas the germination inhibition was statistically significant in the 100 mg.ml⁻¹ treatment. It was observed that the 200 mg.ml⁻¹ treatment completely inhibited the seed germination of Bg 358. The effect of the three concentrations on the germination of Bg 360 seeds was somewhat different from the other two rice cultivars tested. While showing a no significant effect on seed germination by 25 mg.ml⁻¹, the 50 and 100 mg.ml⁻¹ concentrations inhibited the germination of Bg 360 seeds significantly, and the 100% germination inhibition was observed with the 200 mg.ml⁻¹ concentration.

Table 4. Effect of the DSPE on the three rice cultivars and the three rice weeds on soil.

Concentration (mg.ml ⁻¹)	Percentage of inhibition				
	Bg 305	Bg 358	Bg 360	<i>I. rugosum</i>	<i>I. aquatica</i>
Control	36.7±1.7 ^a	5.0±1.3 ^a	35.8±2.0 ^a	40.8±1.5 ^a	27.5±3.4 ^a
25	45.8±2.4 ^a	14.2±1.5 ^a	35.8±2.0 ^a	60.0±9.1 ^b	35.8±1.5 ^a
50	82.5±1.7 ^b	22.5±2.1 ^a	81.7±1.1 ^b	91.7±1.1 ^c	52.5±1.1 ^b
100	100 ^c	83.3±4.0 ^b	95.8±1.5 ^c	95.8±1.5 ^d	97.5±1.1 ^c
200	100 ^c	100 ^d	100 ^d	100 ^e	100 ^d

Note: Values are means of six replicates ±SE, each with twenty seeds. Values in a column with the same superscript are not significantly different (p>0.05).

E. crus-galli seeds in the control started to germinate on the 2nd day. 25, 50, 100 and 200 mg.ml⁻¹ treated seeds inhibited the germination with a significant difference (p<0.05) compared with the control. The inhibitory effect of 100 mg.ml⁻¹ treatment was higher than that of the 25 and 50 mg.ml⁻¹ treatments. The 200 mg.ml⁻¹ treatment was able to inhibit the seed germination of *E. crus-galli* completely. Compared with the control, all four treatments inhibited the seed germination of *I. rugosum* with a significant difference. The highest inhibition was observed in the 100 mg.ml⁻¹ treated *I. rugosum* seeds, whereas the 200 mg.ml⁻¹ treatment completely inhibited the seed germination. There was no significant difference in inhibition between the control and the 25 mg.ml⁻¹ treated *I. aquatica* seeds, whereas the effect of germination inhibition by the 50, 100 and 200 mg.ml⁻¹ treatments was statistically significant. The highest inhibition was observed in 100 mg.ml⁻¹, while the 200 mg.ml⁻¹ treatment completely inhibited the seed germination of *I. aquatica* seeds.

DISCUSSION AND CONCLUSION

The inhibitory effect of *C. urens* seed pericarp was tested against the three rice varieties, Bg 305, Bg 358 and Bg 360, and three rice weeds, *Echinochloa crus-galli*, *Ischaemum rugosum* Salisb. and *Ipomoea aquatica* Forssk. The seeds with the viability above 80% only were selected for germination experiments. Most of the previous studies on seed germination inhibitory effects of plant extracts have used water extracts (Junttila 1997, Chung & Miller 1995) because of the fact that most of the plant substances are water soluble. Therefore in this study too, the water was used to extract the contents of the *C. urens* seed pericarp followed by freeze drying.

The methanol extracts have also been used in some studies. Using lettuce and red beet seeds, Junttila (1997) studied about the germination inhibitors in methanol extracts of red beet fruits (*Beta vulgaris* cv. rubra). The

filter paper method, one of the easiest ways to observe the seed germination in the laboratory has been used to observe seed germination (Gressel & Holm 2006).

The methanol extract of *C. urens* pericarp significantly reduced the seed germination of *E. crus-galli* than the water extracts, indicating that the concentration of the inhibitory substance/s in *C. urens* pericarp was higher in methanol extracts. Thus, the inhibitory action of methanol extracts was increased even at low concentrations. In the light of these observations, further experiments using the methanol extracts were conducted to understand the effect of germination inhibition of the rice and the weed seeds tested.

The germination of all rice cultivars and weed species tested were completely inhibited by 100 and 200 mg.ml⁻¹ concentrations in this experiment. To test the inhibitory effect of *C. urens* on dicotyledons, *Ipomoea aquatica* was used. A complete inhibition of *I. aquatica* seed germination was also observed at 200 mg/ml, suggesting that the *C. urens* seed pericarp has a seed germination inhibitory effect on all weeds tested irrespective of monocotyledons or dicotyledons. In general, the germination inhibitory effects were maximal at high concentrations than at lower concentrations. In this study, it was revealed by the tetrazolium test that the viability of all the weed and rice had not been affected by the DSPE. According to the results obtained, there is an inhibitory effect on the germination of both rice and associated weeds, but the rice seeds resume germination before the weed seeds. Therefore there is a possibility to use the extract of *C. urens* dried seed pericarp to control the rice weeds tested.

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